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Octreotide administration in diabetic rats: Effects on renal hypertrophy and urinary albumin excretion

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Octreotide administration in diabetic rats: Effects on renal hypertrophy and urinary albumin excretion. Initial renal hypertrophy in experimental diabetes is prevented by administration of a long-acting somatostatin analogue octreotide (SMS). To investigate the long-term effects of SMS on renal hypertrophy and urinary albumin excretion (UAE), streptozotocin-diabetic and non-diabetic rats were treated with two daily subcutaneous injections of SMS (100 μg × 2) for six months. Untreated diabetic and non-diabetic animals were used as reference groups. No differences were seen between the two diabetic groups in respect to body weight, food intake, blood glucose levels, urinary glucose output, hemoglobin A_{1c}(HbA_{1c}), fructosamine, serum growth hormone (rGH) or creatinine clearance, but kidney weight (896 ± 36 vs. 1000 ± 24 mg, $P < 0.02$), UAE (417 ± 131 vs. 1098 ± 187 μg/24 hr, $P < 0.02$), kidney insulin-like growth factor I (IGF-I) (167 ± 16 vs. 239 ± 17 ng/g, $P < 0.01$) and serum IGF-I (301 ± 26 vs. 407 ± 17 μg/liter, $P < 0.01$) were all reduced in the SMS-treated diabetic animals when compared to the untreated diabetic group. In non-diabetic rats SMS reduced body weight (274 ± 3 vs. 293 ± 5 g, $P < 0.01$), kidney weight (695 ± 9 vs. 764 ± 17 mg, $P < 0.01$), UAE (83 ± 29 vs. 364 ± 114 μg/24 hr, $P < 0.02$), kidney IGF-I (202 ± 12 vs. 280 ± 12 ng/g, $P < 0.01$), serum IGF-I (428 ± 21 vs. 601 ± 54 μg/liter, $P < 0.01$) and serum rGH (67 ± 6 vs. 126 ± 27 μg/liter, $P < 0.05$) when compared to untreated controls. When kidney weights were expressed in relation to body weight no difference was found between SMS-treated and untreated controls, while the difference between SMS-treated and untreated diabetic animals was still present ($P < 0.01$). In conclusion, chronic administration of SMS has abating effects on diabetic renal hypertrophy and UAE, and thus indicates that SMS may reduce development of diabetic kidney lesions in experimental diabetes. The long-term suppressive effects of SMS on renal enlargement and UAE may in part be mediated through reduction in circulating and kidney IGF-I levels.

A variety of clinical and experimental studies dealing with diabetes mellitus have been designed and conducted in an attempt to characterize the mechanisms underlying the increases in kidney size, glomerular volume and kidney function and the later developing increase in urinary albumin excretion (UAE) and kidney lesions associated with diabetes. The search for significant pathogenic mechanisms in diabetic kidney disease has been focused on the early events in which renal and glomerular hypertrophy and hyperfunction take place. Induction of streptozotocin (STZ)-diabetes in rats causes a rapid increase in renal and glomerular size regularly demonstrable within the first week after the injection of STZ [1–3]. Interestingly, the early renal hypertrophy taking place within the first weeks after induction of diabetes [1–3] is of about the same magnitude as that found after six months [4]. Studies of UAE in experimental diabetes have shown increased values after few weeks of diabetes with a steady increase that continues with diabetes duration [5, 6].

Although growth hormone (rGH) secretion in STZ-diabetic rats is inhibited [7] rather than paradoxically stimulated as seen in human diabetes [8], experiments from our laboratories have suggested a causative role for insulin-like growth factor I (IGF-I) in initial renal hypertrophy in experimental diabetes [9–13]. The initial renal hypertrophy is preceded by a rise in the kidney IGF-I content, which reaches a peak 24 to 48 hours after induction of diabetes [9–13], and strict insulin treatment abolishes both the increase in kidney IGF-I and renal size [9, 13]. Furthermore, administration of a long-acting somatostatin analogue octreotide (SMS) has an equally inhibitory effect on kidney IGF-I accumulation and growth [10] and without affecting the blood glucose levels, supports the hypothesis that renal IGF-I accumulation is a prerequisite for initial diabetic kidney growth.

In the present study we examined the possible long-term effects of SMS on renal hypertrophy, UAE, kidney IGF-I, serum IGF-I and rGH levels in STZ-diabetic rats treated with SMS for six months.

Methods

Study Design

Four groups of adult female Wistar rats (Møllegaards Avlslab., Eiby, Denmark) with initial body weights of 165 to 187 grams were studied. Rats were housed three to four per cage in a room with 12:12 hour (06.00 to 18.00 hours) artificial light cycle, temperature 21 ± 2°C and humidity 55 ± 2%. The animals had free access to standard chow (Altromin # 1324, Lage, Germany) and tap water throughout the experiment. The animals were randomized into four groups; two of these groups were made diabetic by an i.v. injection of STZ (Upjohn Company, Kalamazoo, Michigan, USA) in a dose of 55 mg/kg body weight, under sodium barbital anesthesia (50 mg/kg). Blood glucose was measured 1, 2, 3 and 7 days after the STZ injection, and the urine tested for glucose and ketone bodies by Neostix-4 (Ames, Stoke Poges, Slough, UK). Only animals with blood

glucose levels above 18 mmol/liter, urine glucose concentration above 111 mmol/liter and without ketonuria were included in the study.

One non-diabetic group and one diabetic group were treated with subcutaneous injections of SMS (Sandostatin, Sandoz, Ltd., Basle, Switzerland). The treatment was started immediately after STZ injection and was given in a dose (100 μg twice daily) large enough to maintain high diurnal serum concentrations (that is, above 1000 ng/liter) [10]. Untreated diabetic and non-diabetic control rats were not injected with vehicle but all animals were handled every day and had their cages cleaned.

One week before the experiment started and monthly thereafter the animals were placed in individual metabolic cages for 24-hour urine collections (for determination of urinary glucose, creatinine and albumin excretion) and measurements of food consumption. In addition, body weight and blood glucose were recorded monthly and urine tested for ketonuria. By the end of the study period the animals were anesthetized with sodium barbital (50 mg/kg) and blood was drawn from the retro-orbital venous plexus for hemoglobin A_{1C} (HbA_{1C}) determination and for determination of fructosamine, sodium (Na), potassium (K), creatinine, rGH, IGF-I and insulin in serum. Serum was stored at −80°C until measurements were performed. Furthermore, the left kidneys were rapidly removed and carefully cleaned, weighed, and snap-frozen in liquid nitrogen for later determination of kidney IGF-I content.

Determination of metabolic parameters

Blood and urinary glucose concentration. Blood glucose was measured in tail vein blood by Haemoglucotest 1-44 and Reflolux II reflectance meter (Boehringer-Mannheim, Mannheim, Germany). Urinary glucose concentration was measured in 24-hour urine collections by standard glucose oxidase technique.

Determination of HbA_{1C} and fructosamine. HbA_{1C} was determined using affinity chromatography on columns (Bio-Rad Hemoglobin A_{1C}, Micro Column Test, Richmond, California, USA). Under sodium barbital anesthesia (50 mg/kg) 100 μl blood was withdrawn from the retro-orbital sinus into heparinized tubes and centrifuged. The plasma was separated from the red blood cells immediately and the blood cells were washed in 500 μl 0.154 M NaCl. After lysation in 1 ml distilled water the hemolysate was eluted on resin columns, and both the HbA_{1C} and total hemoglobin concentrations were recorded at 415 nm in a spectrophotometer.

Reagents and standards for the fructosamine assay were purchased from Hoffmann-La Roche, Basle, Switzerland (Fructosamine Test Plus) and the determination performed as previously described [14]. The principle of the assay relies on the reducing potential of ketoamines in alkaline medium. The serum fructosamine assay measurements all glycated serum proteins by forming the corresponding eneaminols, which in turn reduce nitroblue tetrazolium to the colored formazan derivative. The rate of formazane color development correlates with the fructosamine level [14].

Urinary albumin excretion, electrolytes and creatinine clearance

Urinary albumin excretion (UAE). The urinary albumin concentration in 24-hour urine collections was determined by radioimmunoassay as previously described [15] using rat albumin antibody and standards. The urine samples were stored at −20°C until assay was performed. Rabbit anti-rat albumin antibody RARa/Alb was purchased from Nordic Pharmaceuticals and Diagnostics (Tilburg, The Netherlands). For standard and iodination a globulin-free rat albumin was obtained from Sigma Chemical Co. (St. Louis, Missouri, USA).

Measurements of serum sodium (Na), potassium (K), creatinine and urinary creatinine concentration. Serum Na and K were measured using conventional laboratory techniques. Serum and urinary creatinine concentrations (in 24-hr urine collections) were measured by an automated technique adapted from the method of Jaffe and corrected for the prevailing glucose contents (because of interference in the Jaffe reaction). Creatinine clearance was expressed in ml/min.

Hormonal measurements

IGF-I extraction from kidney and IGF-I radioimmunoassay. Kidney IGF-I extraction was performed according to D'Ercole, Stiles and Understood [16] as previously described [9–13]. Briefly, the kidneys were homogenized on ice in 1 M acetic acid (5 ml/g kidney) with an Ultra Turrax TD 25 (Janke-Kunkel GmbH, Stauten, Germany) and further disrupted using a Potter-Elvehjelm homogenizer. The tissues were extracted twice, and, after lyophilization, the samples were redissolved in 40 mM phosphate buffer (pH = 8.0) with 0.2% BSA (Sigma Chemical Co.). Tissue extracts were kept at −80°C until IGF-I assay was performed within two to three weeks after extraction. When kidney extracts were lyophilized and assayed in the presence of biosynthetic IGF-I the mean recovery of added IGF-I was 101% (range 84 to 115). As previously described the kidney IGF-I concentration was corrected for the contribution of entrapped plasma IGF-I [17].

IGF-I antibody UB 286 (raised by L.E. Underwood and J.J. van Wyk, Pediatric Endocrinology, University of North Carolina, Chapel Hill, North Carolina, USA) was donated by the U.S. National Hormone and Pituitary Program. For standard (0.5 to 10 μg/liter) and iodination a full amino acid sequence IGF-I analogue (Amgen Biologicals, California, USA) was purchased from Amersham International (Amersham, Bucks, UK). IGF-I was measured in rat serum after extraction in methanol-acetic acid as previously described [9–13]. Intraassay coefficient of variance (CV) on dublicates was 5%, and interassay CV was 12% (N = 10). The IGF-I antibody has 0.5% cross reactivity with IGF-II and cross reacts minimally with insulin at 10^{-6} M.

Determination of rat GH and insulin. Serum rGH and insulin were measured by radioimmunoassay as previously described [18, 19]. In the rat insulin assay a detection limit of 0.5 mU/liter was achieved by using a two-phase incubation scheme.

Statistical analysis

All results are given as mean values ± SEM. Differences between groups were analyzed by one-way analysis of variance in combination with the Bonferroni test for multiple comparisons and unpaired Student's t-test. Student's t-test for paired samples was used to compare UAE at the beginning and throughout the time course of the study.

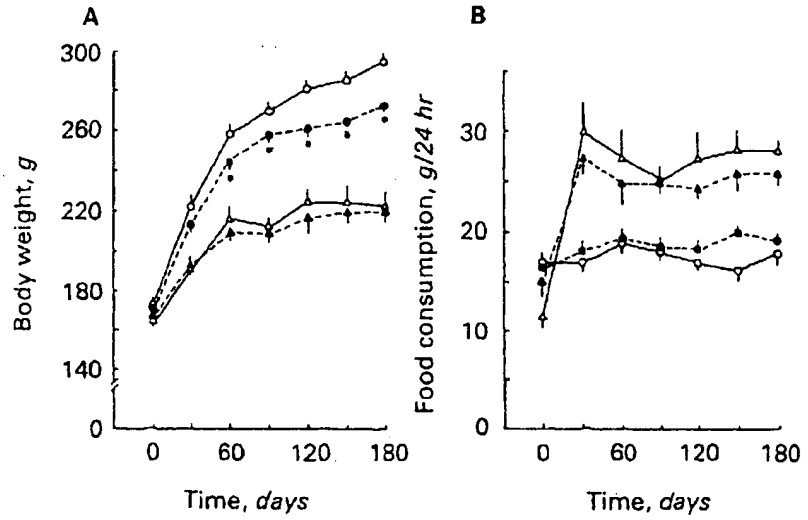

Fig. 1. *Changes in body weight* (A) *and food consumption* (B) *over six months in non-diabetic animals with* (●-----●; *N = 16*) *or without SMS administration* (○—○; *N = 14*) *and in diabetic animals with* (▲-----▲; *N = 10*) *or without SMS administration* (Δ—Δ; *N = 11*). Each point represents mean values with vertical bars denoting SEM. Statistical significance level for differences between SMS treated and untreated non-diabetic animals is indicated by: $^{*}P < 0.01$.

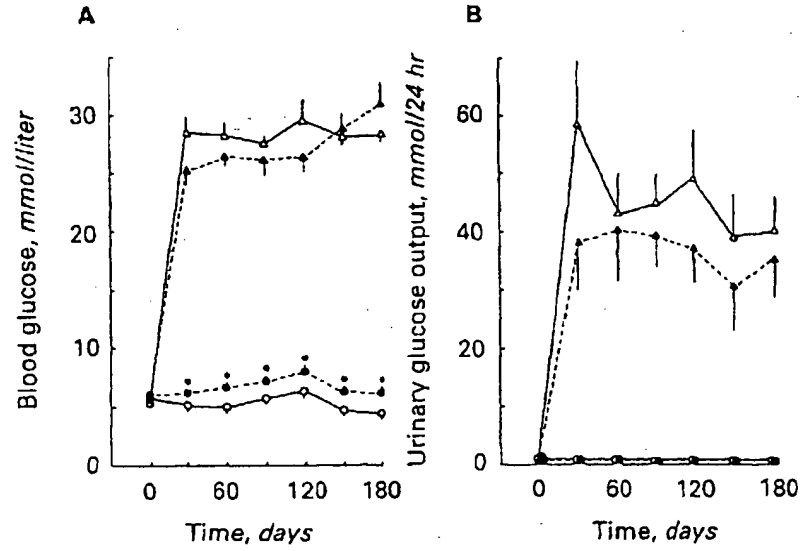

Fig. 2. *Changes in blood glucose* (A) *and urinary glucose output* (B) *over six months in non-diabetic animals with* (●-----●) *or without SMS administration* (○—○) *and in diabetic animals with* (▲-----▲) *or without SMS administration* (Δ—Δ). Data are means ± SEM with the number of animals in the four different groups as indicated in Fig. 1. Statistical significance level for differences between SMS treated and untreated non-diabetic animals is indicated by: $^{*}P < 0.01$.

Results

Body weight and food consumption

Figure 1 shows the monthly measurements of body weight and food consumption in the four experimental groups. As illustrated in Figure 1A, body weight gains in SMS-treated and untreated diabetic animals were reduced compared to non-diabetic groups ($P < 0.01$). However, both diabetic groups grew steadily, albeit slowly, throughout the six months studied (Fig. 1A) and no differences were found at any time between the two groups. In contrast, SMS-treatment of non-diabetic animals induced a body weight gain reduction, statistically significant from month 3 and onwards ($P < 0.01$). Food consumption expressed as g/24 hr was increased by approximately 60% in both diabetic groups when compared to non-diabetic groups (Fig. 1B). SMS-treatment did not affect the food intake in either diabetic or non-diabetic animals (Fig. 1A).

Metabolic control

The blood glucose levels were markedly elevated in the two diabetic groups with mean values between 25 and 30 mmol/liter throughout the study period (Fig. 2A). To be able to estimate an effect of SMS on metabolic control the blood glucose measurements in all groups were performed approximately one hour after the subcutaneous SMS injection. No differences were found in blood glucose levels between the two diabetic groups, however, in the SMS-treated non-diabetic animals a small but consistent elevation in blood glucose concentrations was seen one hour after the SMS injection (Fig. 2A). However, this effect

Table 1. Hemoglobin A_{1c} (HbA_{1c}) and serum fructosamine after six months in control and diabetic animals with or without SMS administration

Experimental groups	HbA_{1c} %	Fructosamine μmol/liter
Controls (N = 14)	1.4 ± 0.1	260 ± 11
Controls + SMS (N = 16)	1.4 ± 0.1	268 ± 7
Diabetic (N = 11)	2.2 ± 0.1*	321 ± 8*
Diabetic + SMS (N = 10)	2.3 ± 0.2*	323 ± 14*

Values are mean ± SEM.
* $P < 0.01$, compared to non-diabetic control groups

on blood glucose levels was not detectable ten to twelve hours after the SMS injection (data not shown). Figure 2B shows the monthly measurements of the urinary glucose output. Undetectable urinary glucose concentrations were found in the non-diabetic groups, and no differences were found in the urinary glucose excretion between the SMS treated and the untreated diabetic groups (Fig. 2B) with mean values around 40 mmol glucose/24 hr. SMS treatment did not affect urine production over 24 hours in either non-diabetic or diabetic animals at any time throughout the study period (data not shown). HbA_{1c} and serum fructosamine levels by the end of the study in the four experimental groups are given in Table 1. Marked elevations were found in both parameters in the two diabetic groups amounting to mean increases of 60% and 22%, respectively, when compared to non-diabetic control groups (Table 1). Furthermore, SMS treatment had no effect on HbA_{1c} or fructosamine levels in either of the treated groups when compared to untreated groups (Table 1). The recorded HbA_{1c} levels are lower than those observed in healthy and diabetic humans, but in accordance with a previous report in mice [20].

Kidney weight

Figure 3 gives the kidney weights after six months with or without SMS treatment in the four experimental groups. The kidney weight increase in the untreated diabetic group amounted to 31% when compared to non-diabetic controls (1000 ± 24 vs. 764 ± 17 mg, $P < 0.01$). The kidney weight increase was diminished in the SMS-treated diabetic animals when compared to untreated diabetic animals (896 ± 36 vs. 1000 ± 24 mg, $P < 0.02$) and amounted to only 17% when compared to non-diabetic controls ($P < 0.01$). In addition, the kidney weight was reduced in the SMS treated controls when compared to untreated controls (695 ± 9 vs. 764 ± 17 mg, $P < 0.01$). However, when the kidney weights were expressed as kidney weight/body weight no difference was found between SMS treated and untreated controls (0.0025 ± 0.0001 vs. 0.0026 ± 0.0001, NS), while the difference between SMS treated and untreated diabetic animals was still present (0.0040 ± 0.0001 vs. 0.0044 ± 0.0001, $P < 0.01$).

Urinary albumin excretion

Monthly consecutive values for 24 hour UAE in the four experimental groups are depicted in Figure 4. Non-diabetic control animals exhibited minimal UAE at the beginning of the study, but developed as previously described [6, 21] a modest degree of age-related increase in urinary albumin excretion during study ($P < 0.01$ Fig. 4). Untreated diabetic animals exhibited a marked elevation in UAE which significantly increased ($P < 0.01$) as early as one month after induction of diabetes followed by a slower further increase over time (Fig. 4). Throughout the whole study period UAE in the SMS-treated diabetic group remained significantly lower when compared to the untreated diabetic rats, with values comparable to untreated non-diabetic control rats (Fig. 4). In addition, SMS-treatment in non-diabetic rats totally abolished the age-related increase in UAE (Fig. 4).

Serum electrolytes and kidney function

Table 2 shows serum sodium (Na), potassium (K), creatinine levels and creatinine clearances by the end of the study. No differences in serum Na or creatinine were found between the four groups, while serum K was decreased in the untreated diabetic group when compared to both SMS treated diabetic and non-diabetic groups (Table 2). Both diabetic groups exhibited increased creatinine clearance when compared to their respective non-diabetic control groups and no differences were found between SMS treated and untreated groups (Table 2).

Serum rGH, insulin, IGF-I and kidney IGF-I

Table 3 shows serum rGH, insulin, IGF-1 and kidney IGF-I levels in the four experimental groups by the end of the study. It has been shown previously that barbital anesthesia induces an increase in GH levels in rats lasting longer than 90 minutes [22], and it follows, therefore, that the rGH levels given in Table 3 are stimulated values. Reduced rGH levels amounting to 48% were seen in response to the diabetic state *per se*, when comparing untreated diabetic animals to untreated controls (Table 3). In addition, a reduction in rGH levels to values comparable to those seen in untreated diabetic animals (Table 3) was seen in response to SMS treatment of non-diabetic animals. In SMS-treated diabetic animals there was a tendency to decreased rGH levels when compared to untreated diabetic animals, but the difference did not reach statistical significance (Table 3). The changes in serum IGF-I levels in the four experimental groups followed the same pattern as that seen in serum rGH. SMS treatment in non-diabetic animals reduced serum IGF-I by 29%, to levels comparable to the decreased levels induced by the diabetic state itself (Table 3). SMS treatment in diabetic animals, however, caused a further reduction in serum IGF-I amounting to 26% when compared to untreated diabetic animals (Table 3). Kidney IGF-I levels by the end of the study (expressed as ng/g kidney) are shown in Table 3. The concentrations shown are all corrected for contribution of plasma IGF-I (Methods). SMS treatment in non-diabetic animals reduced kidney IGF-I by 28% (from 280 ± 13 to 202 ± 12 ng/g, $P < 0.01$; Table 3). Despite the reduction observed in serum IGF-I in untreated diabetic animals, no difference was found in kidney IGF-I levels between non-diabetic and diabetic animals. However, when diabetic animals were treated with SMS a reduction amounting to 30% was seen (239 ± 17 vs. 167 ± 16 ng/g, $P < 0.01$) when compared to untreated diabetic animals (Table 3). Finally, Table 3 gives the serum insulin levels by the end of the study in the four experimental groups. As expected, very low insulin levels were found in both diabetic

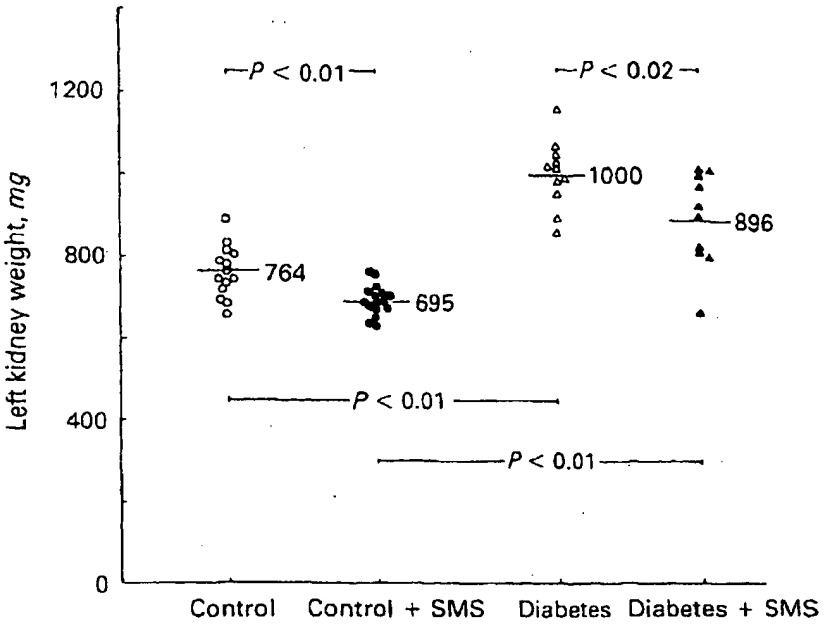

Fig. 3. *Individual kidney weights in each of the four groups by the end of the study.* Mean values for each group are indicated by a horizontal bar. The number of animals in the different groups are as indicated in Fig. 1. Statistical significance levels for differences between the different groups are as indicated in the Figure.

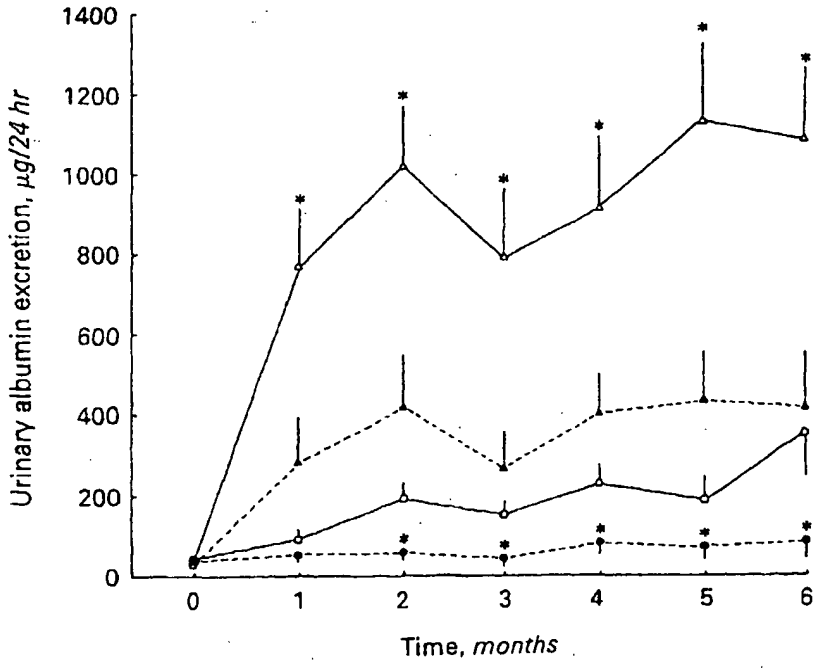

Fig. 4. *Changes in urinary albumin excretion over six months in non-diabetic animals with (●-----●) or without SMS-administration (○——○) and in diabetic animals with (▲-----▲) or without SMS-administration (△——△).* Data are means ± SEM and the number of animals in the four different groups are as indicated in Fig. 1. *$P < 0.05$. SMS treated non-diabetic animals compared to all other groups and untreated diabetic animals compared to all other groups.

groups when compared to the values seen in non-diabetic animals, and it is noteworthy that there was no detectable effect of SMS on the insulin levels (Table 3).

Discussion

The suggestion that SMS may have sustained action on kidneys in long-term diabetic animals arose from the previous observation that SMS treatment abolishes the early transient increase in kidney IGF-1 concentration as well as the initial diabetic renal hypertrophy seen in short-term diabetes [10, 23]. The present study demonstrates reduction in diabetic renal hypertrophy, serum IGF-1, kidney IGF-1 and maintenance of normal UAE in diabetic rats treated with SMS for six months.

The animals in the present study received SMS doses twice

Table 2. Serum sodium (Na), potassium (K), creatinine and creatinine clearance after six months in control and diabetic animals with or without SMS administration

Experimental groups	Na	K	Creatinine	Creatinine clearance
	mmol/liter		μmol/liter	ml/min
Controls (N = 14)	145 ± 4	4.4 ± 0.1	53 ± 2	0.70 ± 0.10
Controls + SMS (N = 16)	149 ± 2	4.6 ± 0.1	53 ± 2	0.55 ± 0.11
Diabetic (N = 11)	144 ± 2	4.0 ± 0.1[a]	57 ± 3	1.10 ± 0.08[b]
Diabetic + SMS (N = 10)	146 ± 2	4.5 ± 0.1	55 ± 2	1.00 ± 0.11[b]

Values are mean ± SEM.
[a] $P < 0.05$, compared to all other groups
[b] $P < 0.05$, compared to non-diabetic control groups

Table 3. Serum rat growth hormone (rGH), serum insulin-like growth factor I (IGF-I), kidney IGF-I and serum insulin after six months in control and diabetic animals with or without SMS administration

Experimental groups	rGH	IGF-I	Kidney IGF-I	Insulin
	μg/liter		ng/g kidney	mU/liter
Controls (N = 14)	125 ± 27[a]	601 ± 54[b]	280 ± 13[c]	10 ± 5
Controls + SMS (N = 16)	67 ± 6	428 ± 21	202 ± 12	14 ± 3
Diabetes (N = 11)	65 ± 10	407 ± 17	239 ± 17	0.4 ± 0.3[c]
Diabetes + SMS (N = 10)	44 ± 8	301 ± 26[b]	167 ± 16[d]	0.3 ± 0.2[c]

Values are means ± SEM.
[a] $P < 0.05$ and [b] $P < 0.01$, compared to all other groups
[c] $P < 0.01$, compared to SMS-treated controls and SMS-treated diabetic animals
[d] $P < 0.01$, compared to untreated controls and diabetic animals
[e] $P < 0.01$, compared to non-diabetic controls

daily large enough to maintain high diurnal serum levels [10]. The persistent effects of the SMS treatment are evident from the suppressed serum rGH and IGF-I levels and from the reduced kidney IGF-I concentrations observed in the SMS treated groups at the end of the study. Furthermore, we observed no toxic (such as, anorexic) effect of SMS treatment, that is, no influence on body weight (only diabetic animals), food consumption, metabolic control or mortality. The fact that we did not find a further growth retardation in SMS-treated diabetic animals is possibly due to the pronounced decrease in serum rGH already induced by the diabetic state *per se*. No measurable effect of SMS treatment was seen on food consumption, serum insulin, urinary glucose output, HbA_{1c} or serum fructosamine indicating that the effect of SMS on the carbohydrate metabolism is negligible.

In accord with previous studies, untreated diabetic rats in the present study exhibited marked and progressive albuminuria and renal hypertrophy within a diabetes duration of six months [4–6]. In sharp contrast to these findings SMS treatment in diabetic rats was associated with a smaller increase in UAE with time, never reaching values significantly different from values in non-diabetic controls. SMS also induced a pronounced reduction in the increase in UAE seen in non-diabetic rats with advancing age. This implies that the effect of SMS observed in diabetic rats does not necessarily ameliorate the functional aberration specific for diabetes. In the present study SMS treatment diminished the final kidney weight in diabetic animals, but it did not completely prevent renal hypertrophy. When kidney weights were expressed in relation to body weight the SMS induced reduction in kidney size observed in non-diabetic rats disappeared. This was not the case for diabetic animals, which may indicate that SMS abolishes only the diabetes related kidney growth. While UAE and renal size were diminished in SMS-treated diabetic rats the metabolic control and food consumption throughout the study remained at identical stable high levels in both diabetic groups. HbA_{1c} and fructosamine at the end of the study were also essentially identical in the diabetic groups, and well in excess of values seen in both control groups. Thus the observed differences between SMS treated and untreated diabetic groups regarding UAE and renal hypertrophy could not be attributed to improved metabolic control in the former.

Very similar observations have been made in STZ-diabetic rats receiving long-term treatment with ACE inhibitors [24], but it is not known whether this treatment has any effect on IGF-I levels, circulating or local. It is interesting that neither treatment seemed to influence metabolic control as demonstrated here for SMS, because it is firmly established that glycemic control is the prime responsible factor in development of diabetic kidney hypertrophy and hyperfunction [25, 26]. So it is suggested that SMS (and ACE inhibitors) play their roles at some later step(s) in the partly unknown sequence of events initiating and maintaining diabetic kidney hypertrophy.

Clinical studies in healthy and diabetic subjects have demonstrated that both native somatostatin [27] and SMS [28] have impressive acute suppressive effects on renal function [glomerular filtration rate (GFR) and renal plasma flow (RPF)]. SMS might exert the same effects on hemodynamics in diabetic rats, but to our knowledge no reports on this topic have been published. However, in a recent paper short-term somatostatin infusion to non-diabetic animals did not affect renal function [29]. Furthermore, SMS treatment in the present study did not reduce the diabetic renal hyperfiltration after six months as far as can be judged from creatinine clearance determinations. Creatinine clearance was employed in the present study as a measurement of kidney function, because the reliable constant infusions technique was feared to reduce the quality of materials to be used for hormonal analysis.

Several hormonal factors have been proposed to be implicated in the hypertrophy-hyperfiltration syndrome of diabetes. Glucagon has recently received interest because imperfectly controlled experimental [30] and human diabetes [31] is characterized by elevated plasma glucagon levels. Furthermore, glucagon administration to normal and diabetic subjects [32, 33] and rats [34] is followed by an increase in GFR. It is interesting that the acute suppressive effect of SMS on renal function in diabetic humans correlated to the decrease in plasma glucagon [28], but it is unknown whether either of these relationships persist in more chronic conditions. In addition to these effects of glucagon on kidney function a relationship between the early increase in plasma glucagon and renal size in diabetic rats has been suggested [35]. However, in other experiments in strictly

metabolically controlled diabetic rats [36] directed at elucidating the role of diabetic hyperglucagonaemia in protein wasting, glucagon administration for up to four weeks to achieve diabetic portal glucagon levels did not promote kidney growth, indicating that glucagon does not play a major role in diabetic kidney hypertrophy.

GH and IGF-I have also been increasingly implicated as possible mediators of diabetic renal hypertrophy and function. The infusion of IGF-I in animals [29] and humans [37] has been shown acutely to increase both GFR and RPF, pointing to a direct effect of IGF-I on kidney function independent of primary changes in kidney size. In addition several studies have shown that IGF-I in hypophysectomized [38], dwarf [39] and pituitary intact animals [40] within days to weeks is capable of inducing renal growth. The existence of 1) distinct receptors for IGF-I in renal glomeruli and tubules [41, 42] and 2) IGF-I messenger RNA expression in all parts of the nephron [43] indicates that IGF-I may affect the kidney in both an endocrine and autocrine/paracrine fashion. In a novel approach, it was shown that transgenic expression in non-diabetic mice of GH or IGF-I for 14 to 37 weeks induced enlarged kidney and glomerular size [44, 45], and in those expressing GH mesangial growth and glomerular changes occurred, claimed to be similar to those seen in diabetic glomerulosclerosis. Whether or not IGF-I was locally accumulated in the kidneys from these animals was not investigated, but it is not unlikely as we have observed that the kidney growth of hypophysectomized rats treated with GH is preceded by a transient increase in renal IGF-I content [46]. The same phenomenon of a transient increase in kidney IGF-I content is seen in initial diabetic hypertrophy, and there is increasing evidence that this very early local IGF-I accumulation is a prerequisite for diabetic renal hypertrophy [9–13]. Furthermore, as mentioned above, both the kidney IGF-I accumulation and the initial renal hypertrophy in diabetic animals are abolished by SMS administration [10]. In the present study, serum IGF-I levels were 29% lower in untreated diabetic animals in comparison to non-diabetic controls at six months. This decrease in serum IGF-I is usually observed in untreated experimental diabetes of more than one weeks duration. SMS administration in non-diabetic animals induced a 29% decrease in serum IGF-I and a further 26% decrease in diabetic animals. In addition, SMS administration reduced kidney IGF-I levels by 28% in non-diabetic and 30% in diabetic animals, in comparison to their respective controls. As GH secretion is already drastically reduced in untreated diabetic rats [7], this confirms previous suggestions that SMS directly inhibits IGF-I formation independent of GH [10, 17]. Whether the depressive effect of long-term SMS treatment on serum and kidney IGF-I bears any causal relationship to the effect of SMS on renal size and UAE is unknown. It is intriguing, however, that the effects of SMS on serum IGF-I were observed not only in diabetic but also in non-diabetic animals, as were the effects on renal IGF-I content.

In long-term clinical studies in diabetic subjects SMS treatment has been reported to reduce or normalize microalbuminuria in diabetic patients over a period of six months [47]. Furthermore, reduced renal hyperfunction and renomegaly was seen in a group of Type 1 diabetic patients treated with SMS for a period of three months, without discernible reductions in serum GH, glucagon or HbA_{1c}, but with pronounced reductions in circulating IGF-I levels [48]. These clinical results are largely similar to those obtained in the present study in experimental diabetes.

In conclusion, chronic administration of SMS to diabetic rats has inhibitory effects on diabetic UAE, renal hypertrophy, serum and kidney IGF-I, without affecting metabolic control or renal function. The mechanism of the suppressive effects of SMS on the diabetic kidney changes may be based on effects of the decreased circulating and renal concentrations of IGF-I. The present study and several others suggest that IGF-I is a factor that should be recognized among the many proposed physical, biochemical and hormonal candidates in the development of diabetic nephropathy and that inhibition of its participation may be rewarded.

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